BEHAVIORAL ASPECTS OF PROTEIN INGESTION BY THE BLOWFLY PHORMIA REGINA MEIGEN[1]

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The problem of specific hungers correlated with unique metabolic requirements has been studied extensively in the vertebrates. The greatly augmented sodium chloride ingestion of persons suffering from adrenal insufficiency is a classical example. Aside from other pathological examples, the most widely studied normal instance of specific hungers is that associated with pregnancy.

It is not altogether surprising that deviations from a standard maintenance diet should occur at times of metabolic stress. Specific hungers have long been known to occur among insects during periods of egg development. Many mosquitoes require a blood meal before they can bring their eggs to ripeness. Similarly, many flies need a meal of protein for oviposition.

While much attention has been directed toward the developmental and hormonal aspects of this phenomenon, there have been few investigations into the behavioral background, specifically in answer to such question as: does the insect actually seek sources of protein? does it ingest protein preferentially? Pospíšil (1958) studied the olfactory responses of a number of saprophilic flies to such odors as skatol, which is associated with decaying proteinaceous material. He found that the fly, during the period of egg development, responded positively to skatol even when it had fed to repletion on carbohydrate; at other times the replete fly was unresponsive. Strangways-Dixon (1959, 1961) reported that the ratio of protein and carbohydrate ingestion by *Calliphora erythrocephala* Meigen varied during the reproductive cycle and that protein was ingested in relatively large quantities during early stages of egg growth as compared to low ingestion during the period of yolk formation. Carbohydrate ingestion followed an inverse course.

The studies reported in this paper were directed toward an understanding of the changes in feeding behavior during the reproductive cycle of the fly *Phormia regina* Meigen and the mechanisms underlying them.

Materials and Methods

Three types of feeding experiments were undertaken. First, flies were placed in individual cylindrical cages constructed of Nylon mesh. Each cage measured 2 cm. in diameter and 5 cm. in length. Through the floor of the cage projected the tips of two 5-ml. volumetric pipettes that had been bent into the shape of a J. The pipettes, which were supported by a clamp and base, served as support for the cage. Every 24 hours the volume of fluid ingested from each pipette was measured by refilling with a hypodermic syringe to the original fluid level and then reading the

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volume directly from the syringe. Evaporation controls were run concurrently (Dethier and Rhoades, 1954).

Second, flies which had been fastened to sticks according to the technique of Dethier and Chadwick (1947) were fed to repletion, and the duration of feeding recorded. Since under given conditions there is a constant relation between the duration of feeding and the volume of fluid imbibed (Dethier, Evans and Rhoades, 1956), the amount of fluid taken could be calculated when desired after sample crops had been removed and weighed for calibration.

Third, flies from which the wings had been removed were allowed to run free on horizontal surfaces where they would encounter lines of fluid drawn in concentric circles around them.

Finally, ingestion was studied by the above-mentioned techniques in flies from which either the ovaries, corpus allatum, or median neurosecretory cells had been removed. Surgical procedures when used were those described by Thomsen (1952) and Dethier and Bodenstein (1958).

In addition to studies of ingestion, preliminary electrophysiological measurements of receptor activity were made by recording through the side wall of the chemoreceptive hairs after the method of Morita (1959). The equipment and techniques were those of Wolbarsht and Dethier (1958).

The flies were from a culture maintained in the laboratory since 1947. Originally homogenized liver was the protein employed for testing. Since similar results could be obtained from a 10% solution of Difco Brain-Heart Infusion, this was eventually substituted in all experiments. It contained, in addition to infusions of calf brains and beef hearts, 2.5 g. disodium phosphate, 5 g. sodium chloride, 2.5 g. bactodextrose, and 10 g. proteose peptone per liter of dry material. For some electrophysiological tests a crystalline bovine hemoglobin was employed. Sucrose was the carbohydrate employed.

Results

Patterns of protein ingestion

Since flies were not able to survive longer than a maximum of four days on a diet of protein alone (*e.g.,* liver homogenate, brain-heart extract), their feeding behavior over long periods could be studied only by providing carbohydrate. It was decided to provide separate sources of protein and carbohydrate concurrently rather than to mix the two. The intake of unadulterated protein could thus be studied.

Under these circumstances the volume of protein consumed daily was found to vary markedly over the lifetime of the fly. There was, furthermore, a difference between the feeding patterns of the sexes. Males, whether mated or not, gradually increased their intake from the time of emergence until the fourth to eighth day (Fig. 1). Thereafter the value reached a low level which was sustained until death. The pattern was similar for virgin females (Fig. 2), but the amount of protein consumed was greater. Mated females, on the other hand, laid eggs sometime between the tenth and fifteenth day, and within 24 hours again increased their protein consumption (Fig. 3). The quantity consumed daily by flies that had not laid eggs was always greater than that consumed by flies at any period after they had laid their first batch of eggs.

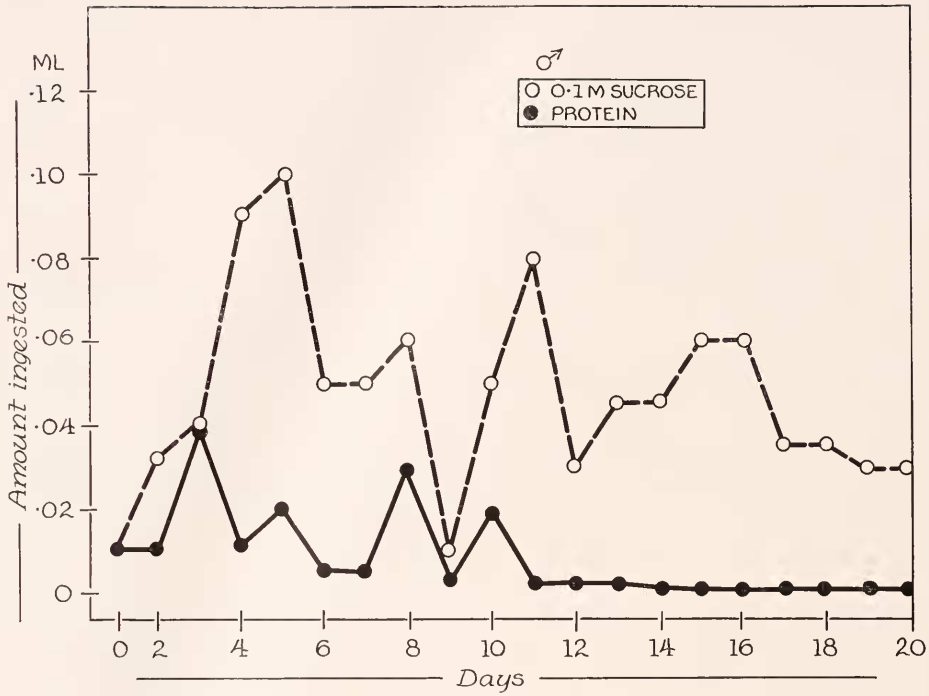

FIGURE 1. Daily intake of 0.1 *M* sucrose and brain-heart extract by a male blowfly in a two-choice situation.

Patterns of carbohydrate ingestion

When carbohydrate (0.1 *M* sucrose) was the only material offered to flies, the pattern of daily intake over the lifetime of the fly was the same for both sexes. Little was taken on the first two days after emergence. Thereafter the intake reached a high value and gradually declined until death some 50 days later for the longest-lived males and 60 days later for the longest-lived females. During this period the daily intake exhibited marked fluctuations, but these were related to differences in activity correlated with variations in the climate of the laboratory. The experiments were conducted under constant lighting but not under constant temperature, humidity, and barometric pressure.

Preferences

If the flies had free access to both protein and carbohydrate at all times from the day of emergence, carbohydrate was nearly always taken in greater volume than protein. If, however, the flies were denied access to protein and maintained on a minimal (0.0001 *M*) carbohydrate diet for the first five days of adult life, the subsequent pattern of carbohydrate and protein intake was quite different. In the case of the males, protein intake remained very low, as before, but carbohydrate intake was very high the first two days (Fig. 4). In the case of virgin females protein

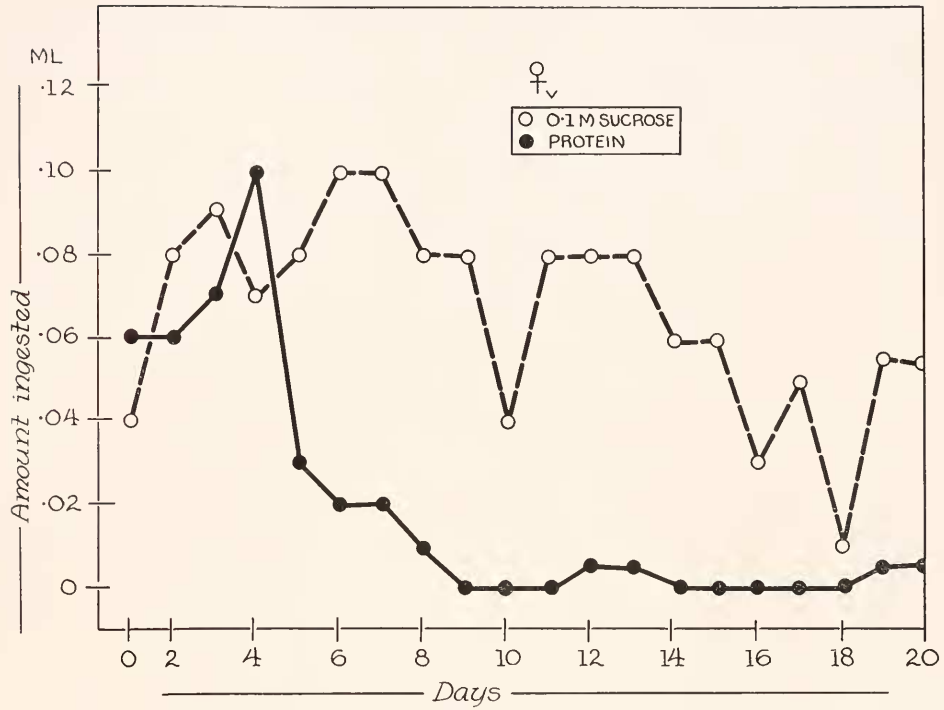

FIGURE 2. Daily intake of 0.1 *M* sucrose and brain-heart extract by a virgin female blowfly in a two-choice situation.

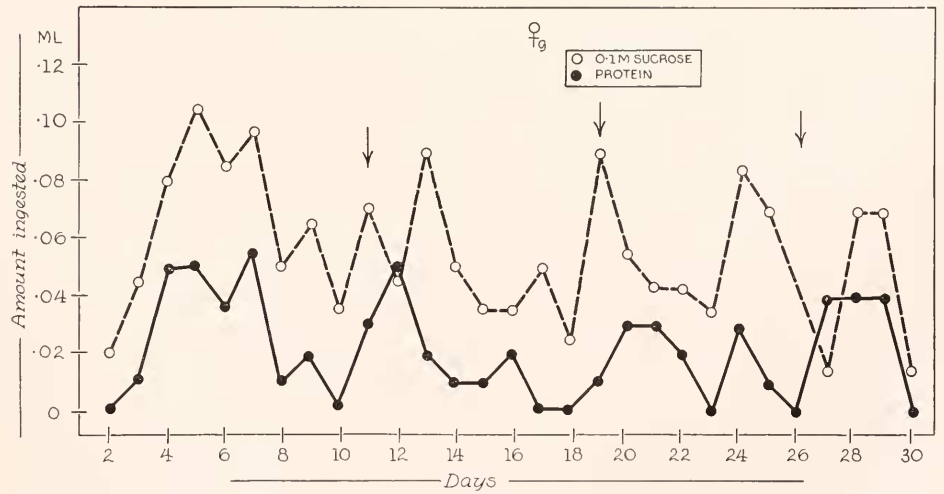

FIGURE 3. Daily intake of 0.1 *M* sucrose and brain-heart extract by a gravid female blowfly in a two-choice situation. Arrow indicate days on which eggs were laid.

intake was very high the first two days and exceeded carbohydrate intake (Fig. 5). If females were denied protein for 10 days, the subsequent preference was even more marked. After 20 days of protein deprivation, a protein preference still existed but was no more pronounced than it had been on the tenth day.

Concentration effects

In the foregoing tests the concentration of sucrose selected (0.1 *M*) was that which flies normally consume in greatest volume over a 24-hour period (Dethier, Evans and Rhoades, 1956). It is not, however, the most highly stimulating con-

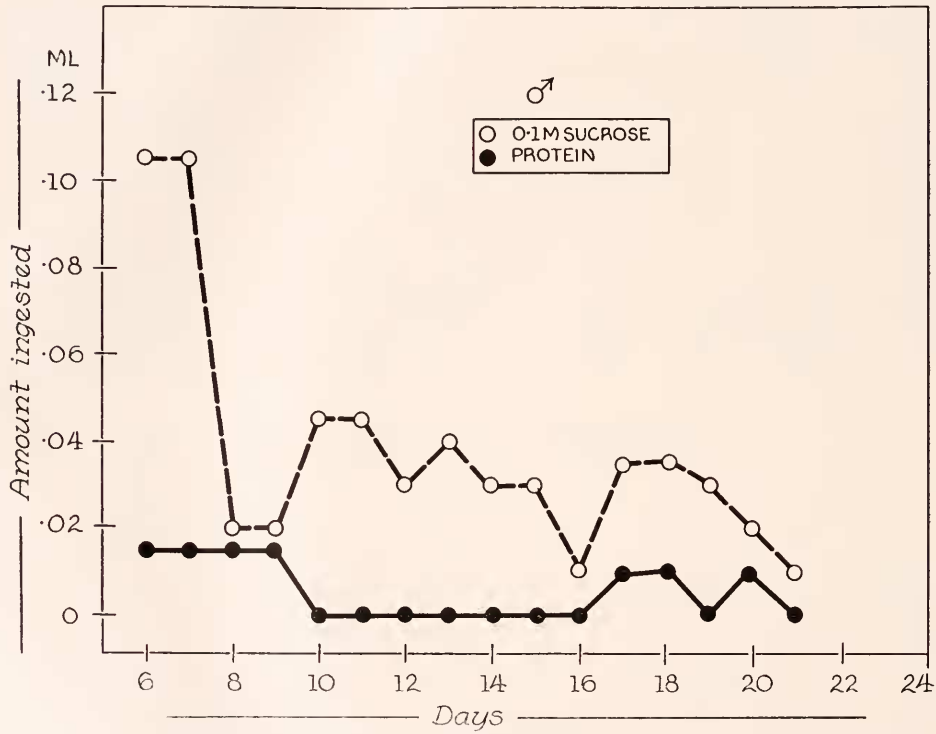

FIGURE 4. Daily intake of 0.1 *M* sucrose and brain-heart extract by a male blowfly in a two-choice situation when it has been deprived of protein for five days.

centration insofar as the sense organs are concerned. Consequently, other series of comparisons were made between protein and various concentrations of sucrose. It is clear from Figure 6 that the availability of a highly stimulating sugar greatly reduced the intake of protein. Conversely, when only a weakly stimulating sugar was available, protein intake was markedly enhanced (Fig. 7).

Initial responses

While the volume consumed has a certain validity as a test of preference, a much clearer insight into the behavior of the fly is obtained by observing its reactions

immediately upon presentation of the different solutions. For this purpose a fly was placed on a sheet of non-absorbent hydrophobic paper and three concentric rings of solution were drawn around it with a camel's-hair brush. In one set of tests the rings from center to outside were in the order water, protein, sucrose; in another set, water, sucrose, protein. Three kinds of flies were tested under these conditions: five-day-old males, starved 24 hours, and previously maintained on 0.1 *M* sucrose; five-day-old virgin females with a similar history; five-day-old virgin females 24 hours starved and previously maintained on protein solution.

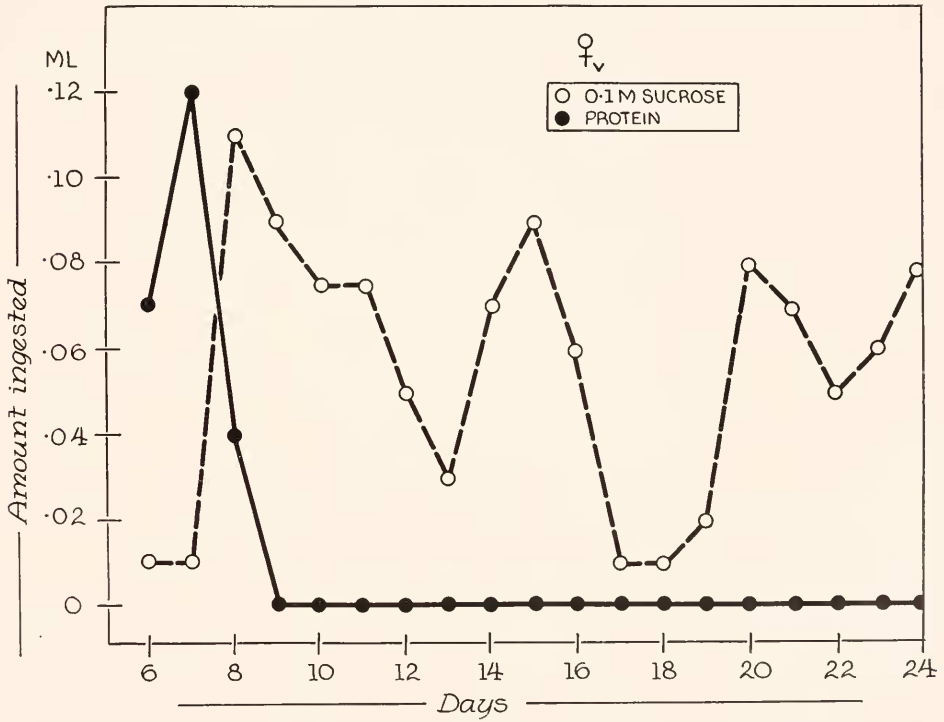

FIGURE 5. Daily intake of 0.1 *M* sucrose and brain-heart extract by a virgin female blowfly in a two-choice situation when it has been deprived of protein for five days.

All flies upon encountering water stopped and drank to repletion; thereupon the water ring became a barrier. Each time a leg encountered the ring the fly turned away where it had previously turned toward the water. This change in response was in itself interesting because it suggested that a solution that was initially acceptable had truly become repellent. Tests in which the water had been absorbed into the paper (thus presenting no unbroken surface) showed, however, that flies after sucking to repletion merely walked across the damp area instead of being repelled. The differences in behavior in the two cases may be explained if we assume that the ring of water stimulated two sets of receptors, the water receptors and mechanoreceptors (the effect of surface tension), the former mediating acceptance, the latter,

rejection. In the thirsty fly the acceptable stimulus overrides the unacceptable one. This balancing of antagonistic stimuli acting on the tarsi and the change in effectiveness with change in internal state is well known (Dethier, 1955; Dethier and Evans, 1961).

Having drunk water, the flies turned away from it. Upon each new encounter they avoided it. After a few minutes, however, their behavior changed. They now waded through and continued until they encountered the next ring of solution. When six-day-old females which had been maintained since emergence on a protein-free diet encountered the protein ring first, they drank the solution avidly, then turned away from it, then followed one of three patterns upon encountering 0.1 *M* sucrose: (1) drank some 0.1 *M*, then ignored it, but would drink 1.0 *M* sucrose if it was presented; (2) ignored 0.1 *M* sucrose, drank 1.0 *M*; (3) ignored all sucrose. If they encountered the sugar ring first, they fed fully on sugar, then drank protein while repeatedly ignoring sucrose. If, however, after drinking protein they were offered 1.0 *M* sucrose, they invariably drank it.

Males maintained on a sucrose diet alone and females which had had protein 24 hours before the test tended to act alike. Encountering protein first they took little or none of it but then drank considerable amounts of sucrose. If they encountered sugar first, they drank a great deal and then virtually ignored protein.

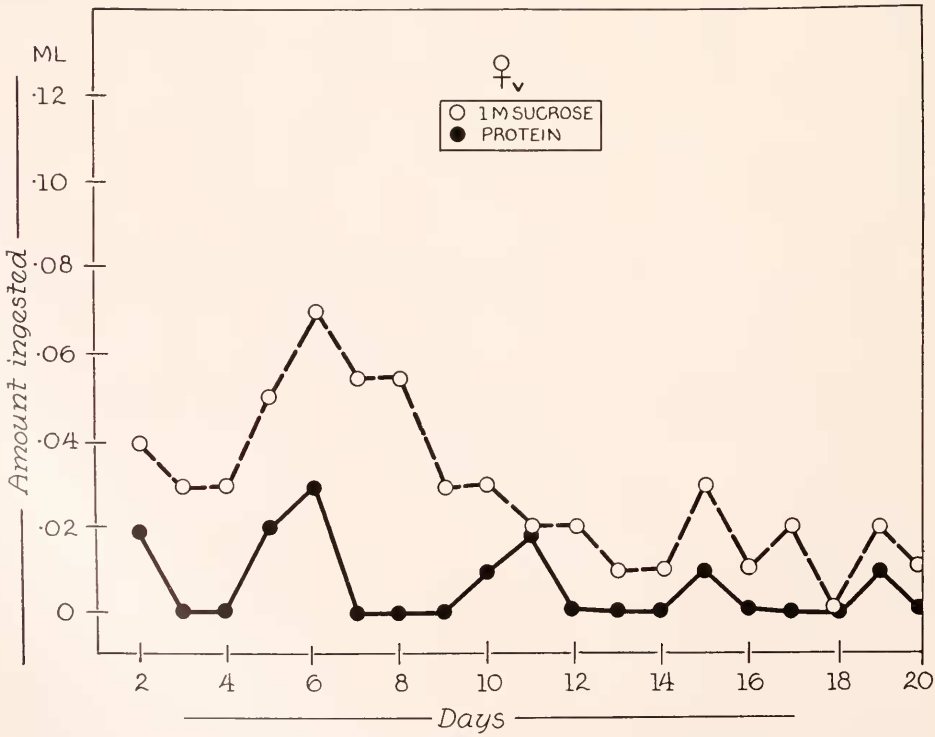

FIGURE 6. Daily intake of 1.0 *M* sucrose and brain-heart extract by a virgin female blowfly in a two-choice situation.

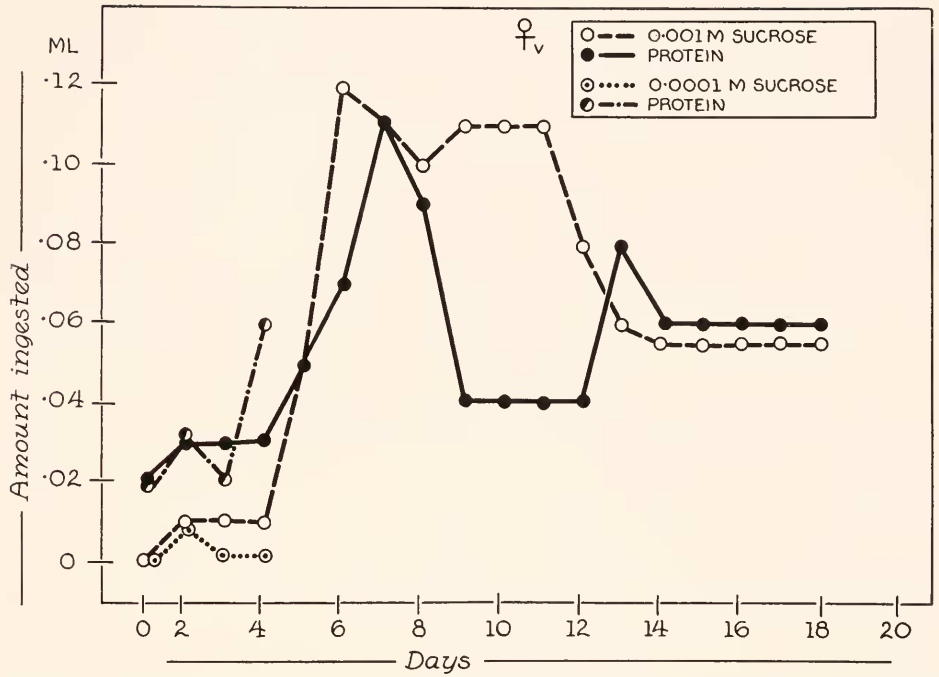

FIGURE 7. Daily intake of sucrose and brain-heart extract by a virgin female blowfly when the protein was paired with 0.001 *M* sucrose and when it was paired with 0.0001 *M* sucrose.

Number and duration of visits in a two-choice situation

In order to assist in correlating the behavior of flies taking single drinks with the measurements of total daily intake, records were made of the number and duration of visits made by flies in the two-choice situation. The records showed that females which had been denied protein made approximately equal numbers of visits to each of the two pipettes but that they took only small nips of the carbohydrate and long draughts of the protein.

Males and females which had had free access to protein also made equal visits to both pipettes but took very few drinks of protein. Drinks of sugar were more hearty.

Anosmic flies

Since all of the protein solutions employed possessed distinct odors, an attempt was made to assess the role of odor by measuring intake and observing the reactions of flies that had been rendered anosmic by removal of the antennae, labellum, and labial palpi. Anosmic flies were tested in all of the situations already described. In no case did their behavior differ from that of the normal flies.

Contact chemoreceptors

It was clear from observing the behavior of flies in the ring tests that they were able to differentiate between protein and sucrose by means of the tarsi and the

labellum. Accordingly, tests were made on the chemosensory hairs of these appendages. Small drops of either protein or 0.1 *M* sucrose were applied to tarsal and labellar hairs of protein-hungry females and the presence or absence of proboscis extension noted. In each case care was taken first to satiate the fly with water and to test each hair first with water. About 40 different labellar and 20 different tarsal hairs were tested. The tests showed that some hairs were sensitive both to protein and to sugar; others, to sugar only. At this time no hairs were found which were sensitive to protein but insensitive to sugar.

Preliminary electrophysiological findings are in accord with these behavioral results, but the matter requires more extensive investigation before the activity of

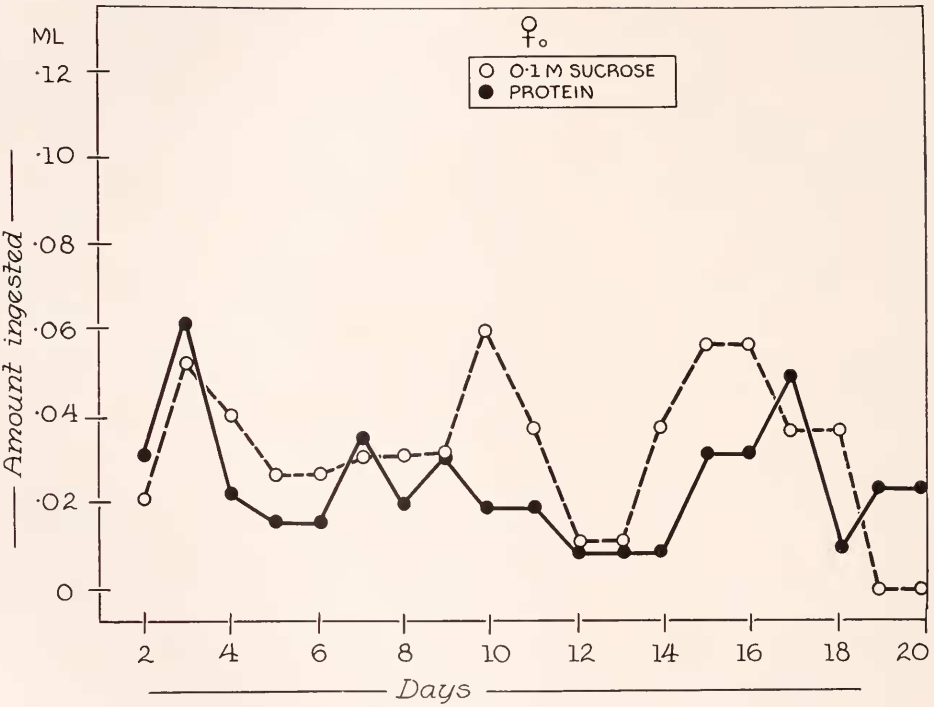

FIGURE 8. Daily intake of 0.1 *M* sucrose and brain-heart extract by a virgin female blowfly from which the ovaries have been removed.

each of the several neurons in the hair is understood. One of the long (ca. 300 μ) marginal labellar hairs tested was sensitive to water, fructose, NaCl, brain-heart extract, and crystalline hemoglobin. Activity was detected in three fibers (Fig. 10). One fiber, as Mellon and Evans (1961) have shown for these hairs, responded to water, one fiber to sugar, and one to sodium chloride. When sugar was applied, both the water fiber and the sugar fiber responded (Fig. 10B). When 1 *M* sodium chloride was applied, the water fiber was suppressed and only the salt fiber responded (Fig. 10D). When a mixture of fructose and sodium chloride was applied, three fibers (water, sugar, and salt) responded (Fig. 10G). When brain-

TABLE I

Total volumes (ml.) of protein and of sucrose taken in the first eight and first 22 days of adult life

Sex and condition	Protein		Sucrose		Total fluid
	8 days	22 days	8 days	22 days	22 days
Virgin female	0.25	0.27	1.08	1.33	1.60
Gravid female	0.26	0.33	1.03	1.37	1.70
Ovariectomized female	0.12	0.19	0.82	1.01	1.20
Female with sham operation	0.17	0.26	0.98	1.10	1.36
Male	0.07	0.09	1.05	1.14	1.23
*Allatectomized female	.12	.29	.25	.84	1.13
*Normal female	.16	.20	.19	.81	1.00
*Female with sham operation	.18	.27	.22	.70	0.97

* Flies in these categories were held for six days before testing. Tests on all other flies began on the day after emergence.

heart extract was added response could be detected in two fibers only (Fig. 10C), but it is not clear at this time which two fibers are responding. In an attempt to clarify this point a mixture of brain-heart extract and fructose was tested and also a mixture of brain-heart extract and sodium chloride. In each case activity could

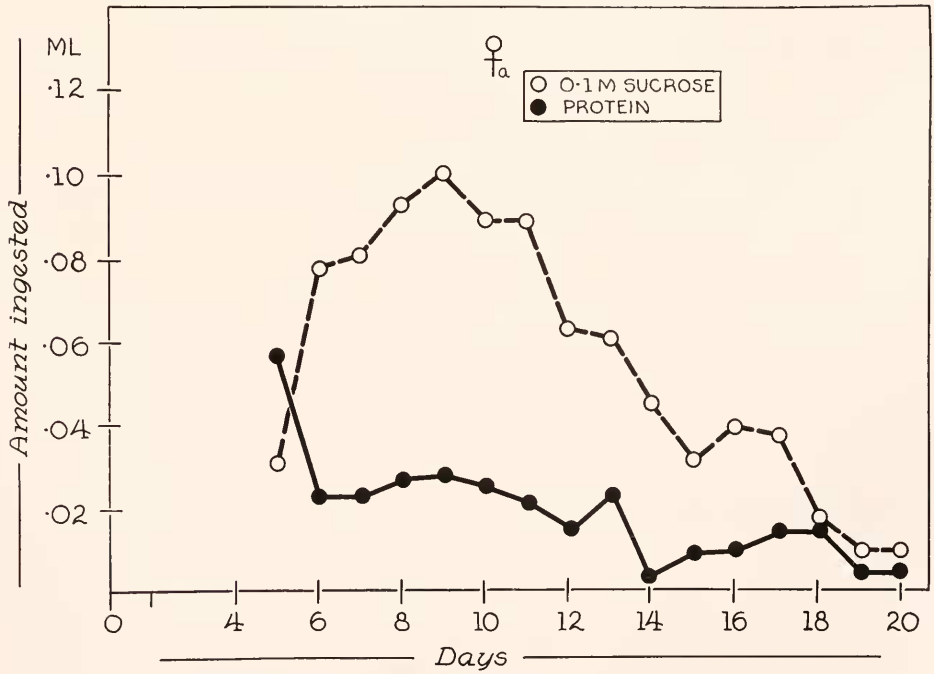

FIGURE 9. Daily intake of 0.1 *M* sucrose and brain-heart extract by a virgin female blowfly from which the corpus allatum has been removed.

be detected in two fibers only (Fig. 10E and F). Crystalline hemoglobin appeared to stimulate only one fiber.

Tests with a medium-sized hair (ca. 100 μ) presented a different picture. This hair responded to water, sugar, and salt; however, when either brain-heart extract or crystalline hemoglobin was applied, all electrical activity was reversibly blocked.

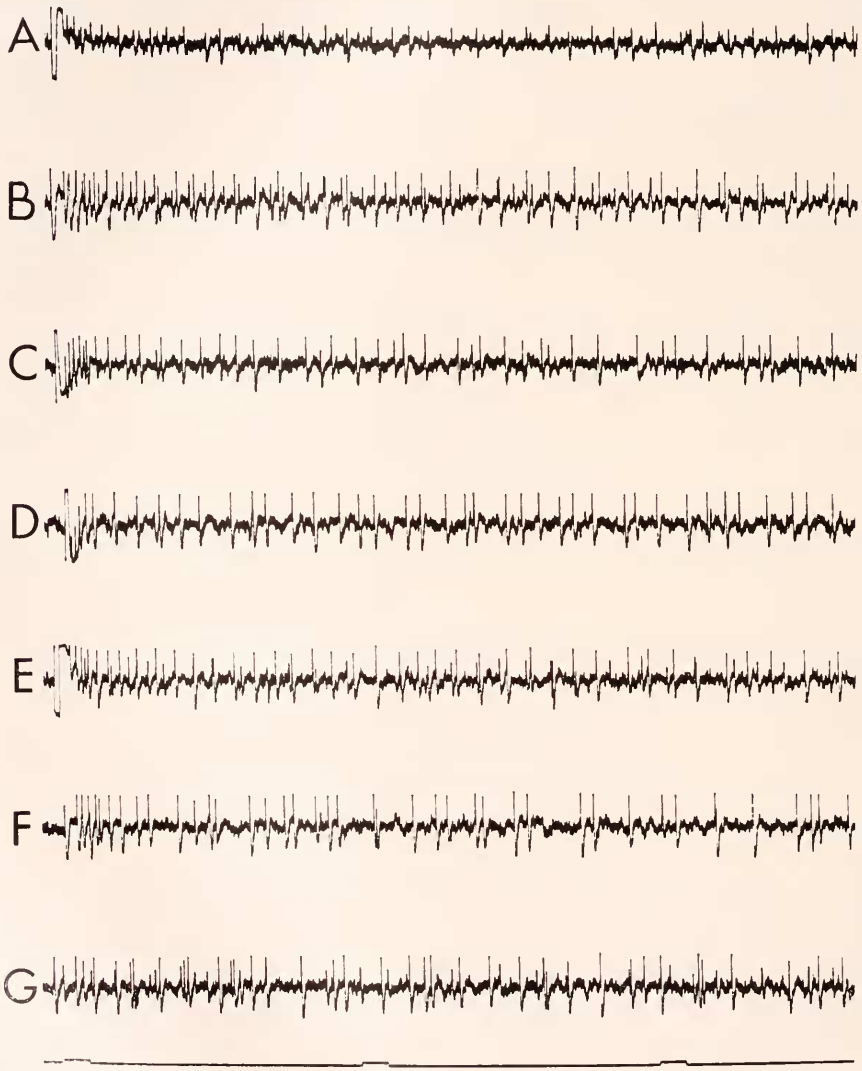

FIGURE 10. Receptor activity recorded through the side wall of one of the large marginal hairs of the labellum of a protein-deprived female *Phormia regina*. A, Response to water. B, Response to 1 *M* fructose. C, Response to brain-heart extract. D, Response to 1 *M* NaCl. E, Response to brain-heart extract plus fructose. Mixture is 0.5 *M* with respect to fructose. F, Response to a mixture of brain-heart extract and NaCl. Mixture is 0.5 *M* with respect to NaCl. G, Response to an equimolar (0.5) mixture of NaCl and fructose. Time, 0.2 second.

Internal factors affecting protein intake

A complicated and still only partially understood hormonal relation exists between the ovaries, the corpus allatum, the corpora cardiaca, and the median neurosecretory cells of the brain. Since preference tests revealed that the pattern of protein intake differs in the male and female and is correlated with ovarian changes, the ovaries and various endocrine organs were removed surgically and protein intake measured.

Removal of the ovaries did not alter the pattern of protein and carbohydrate intake (Fig. 8). Although the total volume of protein taken was less than that of the normals (but not of controls that had had a sham operation), it still showed the typical peak in the first six-day period and still exceeded that of males. There was a slight but not significant decrease in total carbohydrate intake (Table I).

When the corpus allatum was removed, eggs failed to develop; but there was no marked change in the pattern of protein and carbohydrate intake (Fig. 9). The reduction that did occur was matched by that of animals receiving sham operations (Table I).

Flies from which the median neurosecretory cells had been removed failed to survive longer than two weeks. During that period, however, the females exhibited the usual protein peak. Furthermore, in the ring tests they behaved like normal flies in their reactions to protein and carbohydrate.

Discussion

As Strangways-Dixon (1959, 1961) had already demonstrated with *Calliphora,* the volume of protein ingested by female blowflies is greater than that ingested by males and varies with phases in the reproductive cycle. It is clear from studies with *Phormia* that variations in ingestion reflect differences in feeding preferences. Thus, when a fly is given equal opportunity to ingest protein and carbohydrate, it refuses or ingests only a small amount of carbohydrate immediately after emergence and following each period of oviposition. At these times a large volume of protein is taken. The preference is accentuated if the fly is temporarily denied protein during these critical periods.

Observing the behavior of the females deprived of protein one gets the impression that there is a distinct "hunger" for protein. This reaction is particularly interesting because protein by itself is inadequate for survival. Thus, during these periods the fly eschews a nutritionally adequate diet (carbohydrate) for one which meets its reproductive requirements. From an evolutionary point of view, reproduction of the species takes precedence over survival of the individual.

The relationship between protein and carbohydrate ingestion is, however, not absolute. There is a strict dependence on the stimulating effect of the two substances on the sense organs. If, for example, the choice is between a highly stimulating carbohydrate (*e.g.,* 1.0 *M* sucrose) and protein, ingestion is biased in favor of the carbohydrate. Conversely, if the carbohydrate is only weakly stimulating (*e.g.,* 0.001 *M* sucrose), the ingestion of protein is tremendously augmented. Changing the kind of protein also alters the volumes taken. Homogenized liver is preferred to brain-heart infusion and both are preferred to hemoglobin; yet each suffices for egg production (although hemoglobin is the least satisfactory).

At first thought it would appear that the characteristic odors of these materials might be a deciding factor in choice and ingestion. Anosmic flies, however, are no different in their reactions toward protein and carbohydrate than normal flies. This finding is in agreement with data reported by Dethier and Chadwick (1947) and Evans and Barton Browne (1960). The fact that flies can detect the difference between protein and carbohydrate before ingesting them is inescapable. Observations of the behavior toward rings of solutions drawn on paper support the conclusion that discrimination is accomplished through the agency of the contact chemoreceptors on the legs and mouthparts.

Recent behavioral, electrophysiological, and histological work has shown that the innervation of the labellar hairs of *Phormia* is neither so simple nor uniform as originally believed (Dethier and Evans, 1961; Mellon and Evans, 1961; Larsen, personal communication). Some hairs have three neurons; others, four and possibly five. In addition to a neuron responding to bending there is one sensitive to water, one to sugar, and one to monovalent salts. While no specific protein receptor has been found thus far, there are differences among hairs with respect to the protein tested. One type of hair responds to carbohydrate and protein while another type responds only to carbohydrate. Protein appears to block activity in this hair. Both types respond to water and to sodium chloride. Although additional studies will be required before it is possible to assign protein sensitivity to a particular neuron, the point of importance now is that the fly has a peripheral mechanism for protein and carbohydrate discrimination.

Since it is true that the fly's response to protein and to carbohydrate varies concurrrently with events in the reproductive cycle, it must be inferred that the sensory contribution to behavior varies. The two most likely alternatives as to the level where changes occur are: the sense organs themselves; some intermediate level in the central nervous system. Either the relative sensory thresholds to protein and carbohydrate change periodically or some change occurs in the central nervous system where the sensory information is processed. There is as yet no direct information relating to sensory thresholds although by analogy with other sensory modalities in the fly it is unlikely that changes occur here. The alternative is that changes associated with protein metabolism alter integration by the central nervous system of sensory information coming to it. There are a number of ways in which changes occurring in the reproductive cycle might be linked with sensory input.

There are complicated and still only partially understood relations between the endocrine system, the reproductive system, and protein metabolism (*cf.* Wigglesworth, 1954; Strangways-Dixon, 1959). In *Phormia*, as in certain other blowflies, females fed carbohydrate alone are unable to bring the eggs to full development. If protein is provided, eggs develop fully, but they will not be laid unless copulation occurs. If the corpus allatum or medial neurosecretory cells are removed, egg development will progress only as far as in carbohydrate-fed flies.

It is conceivable, therefore, that differences in the fly's behavior toward protein and carbohydrate might be determined by one or more hormones or by changes in protein titer. The two possibilities for endocrine control are that the hormones increase sensitivity to protein or decrease it. If the first alternative is true, removal of the corpus allatum or medial neurosecretory cells should cause changes

in behavior toward protein. Neither changes in behavioral threshold nor difference in intake between operated and normal flies occurred. It might be argued that there was enough residual hormone to prevent a change; however, this appears unlikely since there was obviously not enough to permit egg development. Furthermore, allatectomized flies could be held for eight days on carbohydrate and still show a preference when presented with protein. These experiments rule out any hypothesis that hormones are *directly* concerned with nervous activity leading to protein ingestion.

In no case did removal of any of the endocine glands or of the ovaries prevent the fly from showing a protein peak shortly after emergence. In all cases subsequent protein peaks were absent. Removal of the ovaries alters protein levels simply because there are no developing eggs to create protein demands. Removal of the corpus allatum or medial neurosecretory cells alters protein levels because the absence of hormones also prevents egg development. Additionally, removal of the medial neurosecretory cells interferes with protein metabolism (Thomsen and Møller, 1959). Insofar as protein is concerned Strangways-Dixon (1959, 1961) obtained similar results with *Calliphora*. He did report, however, that allatectomy depressed carbohydrate intake, and this did not occur with *Phormia*.

The occurrence of an initial peak in protein ingestion unaffected by allatectomy, removal of the ovaries, or removal of the medial neurosecretory cells might be explained by assuming that all flies emerge from pupation with a protein deficit and that it affects behavioral threshold. This would explain the occurrence of initial protein ingestion in both sexes. In males the quantity is small and soon approaches zero. In the newly emerged female the initiation of egg development causes withdrawal of protein from the fat body (Strangways-Dixon, 1959) thus increasing the deficit. Accordingly, if females are denied protein for the first six days, the deficit becomes acute and sensitivity should increase (as indeed it does). In the males there is no such increase. Greenberg (1959) stated that there was no difference in the protein consumption of male and virgin female houseflies; however, his conclusion was based upon a comparison of mean daily intake, a measurement which tends to minimize the differences occurring shortly after emergence. After this time there are no pronounced differences. If a female is mated, a protein deficit develops, and the cycle is repeated.

The point at which protein deficit influences sensory input is at present unknown. It is not merely a matter of body pressure or osmotic relations in the body cavity because injection of carbohydrate, water, or hypotonic salt into the hemocoel fails to alter the pattern of protein ingestion. It is unlikely that it is any aspect of gut physiology because transection of the recurrent nerve, which innervates the gut, also fails to alter protein ingestion. When protein-deficient females are placed in a choice situation after recurrent nerve transection, they become hyperphagic, but do so by ingesting carbohydrate rather than protein.

The assistance of Mr. D. Mellon is gratefully acknowledged.

Conclusions

There is a difference in the pattern of protein feeding by the two sexes of the blowfly *Phormia regina* Meigen. Males, whether mated or not, gradually increase

their intake from the time of emergence until the fourth to eighth day. Thereafter little protein is taken. The pattern is similar for virgin females, but the volume ingested is greater. Mated females increase their protein intake after each batch of eggs is laid. If females are denied protein at times when the intake would normally be great, they show a decided preference for protein over carbohydrate in a choice situation. Choice is mediated by the contact chemoreceptors; odor is not a factor. The peak in protein ingestion that occurs after emergence is not altered by removal of the ovaries, corpus allatum, or medial neurosecretory cells. Subsequent peaks are abolished by any procedure that prevents egg development. Changes in feeding behavior are correlated with changes in protein levels which in turn are related to hormonal and reproductive cycles. It appears unlikely that hormones are directly concerned with nervous activity leading to protein ingestion.

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